# OVIPOSITION BEHAVIOUR OF THREE INTRASPECIFIC VARIANTS OF THE VISCERAL LEISHMANIASIS (KALA-AZAR) VECTOR *PHLEBOTOMUS ARGENTIPES* [1]

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(*With one text-figure*)

**Key words**: *Phlebotomus argentipes*, visceral leishmaniasis vector, oviposition behaviour, intraspecific variation

Oviposition behaviour of *Phlebotomus argentipes* Annandale & Brunetti *sensu lato* studied in the visceral leishmaniasis (VL, kala-azar) endemic and non-endemic parts of Tamil Nadu suggests that (i) the species has 3 intraspecific variants (possibly representing separate species), (ii) the mean number of eggs laid per fly is higher in the flies fed on cattle than on human beings, (iii) the variant human-fed form is sympatric with the neighbouring cattle-fed form, while both the cattle fed variants are allopatric in nature, (iv) the possible cause for the vector capacity of the form feeding on humans is discussed, (v) research on the behaviour, based on genetics and using molecular biological tools such as isoenzyme analysis and DNA paw printing, is needed to resolve the taxonomic status of *P. argentipes*.

## Introduction

*Phlebotomus argentipes* Annandale & Brunetti *sensu lato,* described by Annandale (1908), has been studied extensively as a vector of the Indian visceral leishmaniasis (VL, kala-azar). The currently known geographical and biological variations of *P. argentipes* may consist of a complex of sibling species (Seccombe *et al.* 1993), that are morphologically similar but different in behaviour. Recently, two morphospecies found sympatrically were described from the city of Chennai (=Madras) (Ilango *et al.* 1994), one of the known foci of VL.

Incrimination of vector species among the species complex is extremely important for taxonomists before developing any control strategies. However, the problem among closely allied variants in insect vectors is that competition for resources, such as feeding hosts and mating sites, in similar ecological conditions, leads to divergence of behaviour and formation of two or more species (Dobzhansky *et al.* 1976). This is usually found in disease endemic regions. To differentiate such closely related species, morphological taxonomy serves a limited purpose, but molecular techniques and genetics based behavioural studies are extremely reliable. In view of this, while surveying the wetland mosquito fauna of Tamil Nadu, the oviposition behaviour of *P. argentipes* was studied in parts of the state where kala-azar is endemic.

## Study Area

In Tamil Nadu, the city of Chennai and two rural districts, Ramanad and Tirunelveli, known endemic foci of VL, were surveyed for the phlebotomine sandfly fauna during 1987-90. Recently, a few cases of kala-azar were reported from Chennai, but the disease was unknown in the Ramanad and Tirunelveli district. Chennai was, therefore, considered an endemic focus, while Ramanad and Tirunelveli were designated as non-endemic. For the present study, Tirunelveli (peridomestic) and Chennai (domestic and peridomestic) were chosen for

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sample collection because they are widely separated from one another, and represent different habitats. Further, in Chennai, samples were collected from the centre of the city, Royapettah (domestic) where VL was known to exist, and from the suburban village Poonamalle, 30 km west of Chennai, from where VL was not known.

## METHOD

Veeravandiyur village in Tirunelveli district and Poonamalle village near Chennai are identical habitats in their housing pattern, surrounded by cattle sheds, paddy fields and irrigation ponds. Royapettah has typical urban dwellings with crowded housing. Stray cattle were often found on the streets. Humans and cattle are the sources of blood meal for *P. argentipes* prior to oviposition.

As *P. argentipes* is nocturnal, night collection of samples was made from both human dwellings and cattle sheds. In all the 3 study sites, blood-fed females were collected from the abdomen of cattle in Veeravandiyur and Poonamalle villages, humans also served as bait simultaneously. Blood-fed flies were individually stored in ½" x 3" glass tubes and left undisturbed overnight to oviposit. 5% glucose solution soaked in cotton was supplied to each fly as nutritional supplement.

## RESULTS

Fig. 1 shows the locations (ABC) from which the females of *P. argentipes* were collected; the bar diagrams represent the mean no. of eggs laid per fly. The distance between Veeravandiyur (A) and Royapettah (B) or Poonamalle (C) is 680 km and Royapettah (B) and Poonamalle (C) 30 km. In Veeravandiyur and Poonamalle villages, the female flies showed a greater preference for cattle than for human beings, whereas in Royapettah they were equally attracted to both hosts.

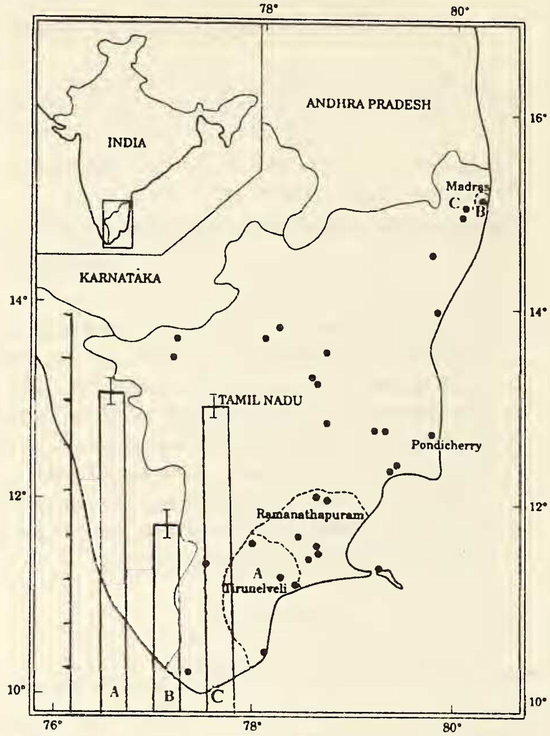


Fig. 1. Locations of three intraspecific female variants (A, B, C) of *P. argentipes* collected. Bar diagrams represent the mean no. of eggs laid per fly

(A) Veeravandiyur village, Tirunelveli
Total no. of flies captured (N) = 39
No. of eggs laid per fly

| | | |
|---|---|---|
| Mean (x) | = | 71.64 ±3.64 (s.d) |
| Range (R) | = | 65-77 |

(B) Royapettah in Chennai
Total no. of flies captured (N) = 21
No. of eggs laid per fly

| | | |
|---|---|---|
| Mean (x) | = | 41.66 ±4.83 (s.d) |
| Range (R) | = | 32-50 |

(C) Poonamalle village near Chennai
Total no. of flies captured (N) = 32
No. of eggs laid per fly

| | | |
|---|---|---|
| Mean (x) | = | 69.00 ±4.46 (s.d) |
| Range (R) | = | 54-76 |

## Discussion

The present study reveals some intriguing biological variations in the oviposition behaviour of *P. argentipes* collected from 3 different places, their host preference and the mean number of eggs laid per fly. The eggs laid by the variants AC are similar in their oviposition sites and mean no. of eggs laid per fly, but both are quite different from B. The ecological distribution of these variants shows that A is allopatric to BC and B sympatric to C. AC are more strongly attracted to a blood meal from cattle than from humans. B is attracted to both cattle and humans, the latter providing a better opportunity to transmit kala-azar.

Several possible explanations can be given for the differences in behaviour and distribution of these variants. For AC, feeding on cattle appears to be more advantageous, as they offer a large, open body surface where the blood meal can be taken immediately after mating. Cattle blood is richer in iron than human blood and perhaps they are less sensitive to biting. Hence, cattle are preferred by AC. Human blood meal is preferred by B, which could be the vector of visceral leishmaniasis.

These observations coincide with the known findings on the two morphologically different species. In the present study, the intraspecific variants AC are similar to the morphological species A, which occurs in Tamil Nadu and the whole of South Asia and has perhaps no role in the transmission of kala-azar. The variant B found in Royapettah, and also reported from other major endemic areas like Bihar and West Bengal, is a morphologically identifiable species B and is considered a vector species.

*P. argentipes* is considered to be a species complex with member species differing in the lengths of the fourth antennal ascoids (Lewis and Killick-Kendrick 1973), of the labrums (Lewis 1987) and in cuticular hydrocarbons (Kamhawi *et al.* 1992). Recently, Ilango (1998) reported differences between the specimens of *P. argentipes* collected from endemic and non-endemic areas of visceral leishmaniasis, in which the relative size of the fourth antennal ascoids shows character displacement. According to Brown and Wilson (1956), character displacement is observed in two closely related species when their allopatric populations are very similar and their sympatric populations distinct in one or more characters. The disparate characters could be morphological or behavioural. In this study, the pattern of distribution and oviposition behaviour of three variants of *P. argentipes* suggests that it may consist of several isomorphic species distributed across the Indian Subcontinent.

According to Tabachnick and Black (1995), current species identification using isoenzyme analysis, DNA probes and PCR delimits species and provides genetic relationships. Molecular taxonomy promises to be an important tool for (1) discrimination of cryptic members of species complexes, (2) identification of morphologically similar species at any life stage, and (3) rapid identification of small arthropods (eg. mites, sandflies, *Culicoides*). Population genetics characterises genetic variation within and among populations of a species. Members of species complexes and morphologically similar species are likely to be descendents of populations that were once members of a single species. Studies that examine gene flow with respect to components of vector capacity provide insights into vector species complexes and variation within species.

Hence, molecular taxonomy and population genetics studies are urgently required to resolve the taxonomic status of *P. argentipes,* to understand the pathogenic transmission, epidemiology and control of the disease.

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